The rebound of lipoproteins after LDL-apheresis. Kinetics and estimation of mean lipoprotein levels

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Received 23 December 1998; received in revised form 10 November 1999; accepted 16 December 1999

Abstract

We studied the rebound of lipoproteins in 20 hypercholesterolemic men [mean total cholesterol (TC) levels 9.6 ± 1.8 mmol/l] after LDL-apheresis (LA) to determine the rate of recovery and the change in cholesterol synthesis, and to find a uniform estimation for time-averaged levels. After 10–20 months on biweekly LA using dextran sulfate cellulose columns and concomitant simvastatin administration, time-averaged levels (± SD) measured by integration of the area under the curve were as follows: TC 4.4 ± 1.0 mmol/l, LDL cholesterol (LDL-C) 2.5 ± 1.0 mmol/l, apolipoprotein B (apo B) 1.3 ± 0.3 g/l, triglycerides (TG) 1.7 ± 0.7 mmol/l, HDL-C 1.1 ± 0.2 mmol/l, and lipoprotein(a) [Lp(a)] 53.7 ± 49.4 mg/dl. Mean acute reductions in TC, LDL-C, apo B, Lp(a), and TG were 61, 77, 75, 76, and 62%, respectively. HDL-C levels were not influenced. Median recovery half times for TC, LDL-C, apo B, and Lp(a) were 3.0, 4.0, 2.3, and 3.5 days, respectively. The rebound of Lp(a) was identical to LDL-C, in 12 and 13 days post-treatment, respectively, whereas apo B and TC returned to pre-treatment levels in 7.5 and 10 days, respectively, due to the fast rebound of VLDL particles. Notwithstanding these differences, time-averaged levels (C AVG) could be estimated uniformly for the four latter parameters with the formula: C AVG = C MIN + 0.73(C MAX − C MIN), where C MAX and C MIN are the immediate pre- and post-treatment levels. During long-term treatment the whole-body cholesterol synthesis was increased as measured by the ratio lathosterol to cholesterol of 3.24 ± 1.49 mmol/mmol, whereas no further transient increase in the recovery period after LA was found. In conclusion, long-term LA and simvastatin therapy induced acute and chronic changes in lipids and lipoproteins showing the feasibility of biweekly treatment. It was shown that time-averaged levels, as a measure for the effective plasma levels, can be accurately estimated from pre- and post-treatment levels only. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Atherosclerosis; Lathosterol; LDL-apheresis; Lipid lowering; Rebound; Sitosterol

1. Introduction

The performance of regular low density lipoprotein (LDL) apheresis permits the achievement of lower levels of LDL cholesterol which is usually not possible to attain with drug therapy alone [1,2]. Consequently, the application of LDL-apheresis may offer opportunities in the arrest of progression or regression of coronary and peripheral vascular disease in selected hypercholesterolemic patient [3–13]. Only recently, two controlled LDL-apheresis regression studies were published, FH Regression Study and LAARS [10,11]. Both studies showed angiographical arrest of progression of coronary artery disease in severe hypercholesterolemic subjects concomitantly treated with an HMG-CoA reductase inhibitor, whereas LAARS also showed reduction of the extent of peripheral vascular disease [14]. Treatment using selective LDL-apheresis removes large amounts of apolipoprotein B (apo B) containing lipoproteins from the body in a relatively short time, generally 2–4 h. Sawtooth-like alterations in lipoprotein concentrations are one of the most striking
differences between patients undergoing repetitive LDL-apheresis and conventional therapy [15]. The efficacy of the treatment depends on the pre- and post-treatment lipid levels, and on the post-treatment return of lipids in plasma [16–20]. The combination therapy of LDL-apheresis and an HMG-CoA reductase inhibitor can be expected to decrease pre-treatment LDL cholesterol to lower levels and slow down the post-apheresis rebound, which permits prolongation of the intervals between the apheresis procedure [17,20]. It has been shown that the increase of lipoprotein levels after the treatment can be explained by first order kinetics [16,21]. Therefore, it is currently accepted that time-averaged concentrations provide the best estimate of the physiologically effective plasma levels of cholesterol during long-term treatment with LDL-apheresis [18].

There is no universal agreement whether the cholesterol synthesis is increased after a single apheresis or during regular treatments. An increased rate of cholesterol biosynthesis without any change in apo B synthesis has been reported after plasmapheresis [22–24], whereas both a transient increase and no change in cholesterol synthesis have been shown after LDL-apheresis [19,20,25,26].

In this paper, we measured the rebound of lipids and lipoproteins in days following a treatment during long-term LDL-apheresis using dextran sulfate cellulose columns (DSC) [27]. Changes in the amounts of lipids and lipoproteins were measured, and the absolute and fractional recovery rates were calculated. Changes in cholesterol biosynthesis and in intestinal adsorption rate were quantitated indirectly by measurements of the sterol intermediate lathosterol and the plant sterol sitosterol in relation to serum cholesterol, respectively. The data were used for estimation of the mean effective lipid or lipoprotein concentrations with a formula during long-term biweekly treatment in the LDL-Apheresis Atherosclerosis Regression Study (LAARS) [11].

2. Patients and methods

2.1. Subjects and treatment

The present study was carried out in subjects randomised to treatment with LDL-apheresis in the LDL-Apheresis Atherosclerosis Regression Study (LAARS) [11]. In this study the effect of 2 years of cholesterol lowering using biweekly LDL-apheresis plus simvastatin treatment was compared with the effect of conventional lipid lowering with simvastatin alone on coronary [11,28] and peripheral vascular disease [14]. All eligible LAARS participants were men with a primary hypercholesterolemia and extensive, angiographically assessed severe coronary artery disease. This study was approved by the ethical committee of the University Hospital of Nijmegen. Twenty-one subjects were enrolled for long-term LDL-apheresis, of whom 20 men gave informed consent for the present study. All subjects, age 50 ± 9 years, range 30–66, were classified as heterozygous for familial hypercholesterolemia. Baseline lipids and lipoproteins before the start of the trial on a standard lipid lowering diet were as follows: total cholesterol 9.64 ± 1.78 mmol/l, triglycerides 2.40 ± 1.04 mmol/l, LDL cholesterol 7.66 ± 1.92 mmol/l, high density lipoprotein (HDL) cholesterol 0.96 ± 0.18 mmol/l, lipoprotein(a) [Lp(a)] 59.7 ± 64.3 mg/dl [median 34.7, range 3.1–236.3], and apo B 2.55 ± 0.43 g/l. Concomitant treatment with the HMG-CoA reductase inhibitor simvastatin (40 mg/day) was started at the beginning of the study. LDL-apheresis was performed fortnightly with an automated system equipped with two small-sized dextran sulfate cellulose columns (Liposorber®) in combination with a membrane type plasma separator (MA-01 unit, Kanegafuchi Chemical Industry Co Ltd, Osaka, Japan). During this extracorporeal procedure blood was anticoagulated with heparin at a rate of 2000 U/h. A volume of 5000 ml (approximately 1.5 plasma volumes) was treated per session, which lasted 3–4 h at a blood pump rate ranging between 80 and 120 ml/min. The present experiments were performed for every subject on one occasion, 10–20 months after the start of the first apheresis.

2.2. Rebound curves

In order to follow the return of the various lipids and (apo)lipoproteins to their pre-treatment values blood samples were taken immediately before and immediately after apheresis, and at 24 h intervals subsequent to the apheresis for a period of 7 days, and on the 10th and 14th day post-apheresis. For construction of the rebound curve additional data of immediate pre- and post-treatment levels from four forgoing apheresis procedures were used to reduce the individual variability. The coefficient of variation of these pre- and post-treatment levels was within total analytical and biological variance.

2.3. Analytical procedures

All blood samples, except those taken immediately after apheresis, were obtained between 08:00 and 09:00 h by venepuncture after an overnight fast. The post-apheresis samples were drawn between 13:00 and 14:00 h. For the indirect assessment of whole body cholesterol biosynthesis in vivo we used the quantification in serum of the cholesterol intermediate lathosterol, and more specifically the lathosterol to cholesterol ratio [29–31]. Serum concentrations of lathosterol are directly associated with hepatic HMG-CoA reductase activity [32]. Serum lathosterol concentration and the lathos-
terol to cholesterol ratio are independent of the composition of diet [30], but are increased during treatment with resins [33] and decreased during therapy with HMG-CoA reductase inhibitors [30,34], reflecting corresponding changes in cholesterol biosynthesis in the liver. Plasma concentrations of the plant sterol β-sitosterol were also measured. Sitosterol levels and especially the sitosterol to cholesterol ratio reflect the balance between sterol absorption and biliary excretion rate, and are closely related to the fractional absorption of dietary cholesterol [35,36]. Plasma lathosterol and sitosterol levels were measured by high-performance liquid chromatography (HPLC Spectra Physics model 8800, Breda, The Netherlands), essentially as reported before, and shown in mmol/l and also as mmol/mmol cholesterol to correct for the effects of apheresis in removing lipoproteins [30]. Plasma cholesterol and triglycerides were determined with commercially available enzymatic methods (Boehringer Mannheim, FRG, Nos. 237574, and Sera-PAK, Miles, Italy, no. 6639, respectively). To determine plasma HDL cholesterol the polyethylene glycol 6000 precipitation method was used [37]. LDL cholesterol was calculated by the Friedewald formula. Samples for apo B and Lp(a) were initially stored at −80°C, and determined at the end of the study. Apo B was quantified by immunonephelometry [38] and recalculated on the basis of data from exchange of sera with the North West Lipid Research Clinic (Seattle, USA). Lp(a) was be measured by a specific radioimmunoassay (apolipoprotein(a) RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden).

2.4. Statistical procedures

Assuming a one-compartment model and semi-steady state conditions, the rate of return to pretreatment cholesterol concentration can be predicted from the equation: 

\[ C_T = C_{MAX} - (C_{MAX} - C_{MIN}) e^{-kT} \]

where \( T \) is the time in days after the treatment, and \( C_{MAX} \) and \( C_{MIN} \) represent the levels immediately pre- and post-apheresis [16]. The first-order turnover constant \( k \) or the fractional catabolic rate (FCR) can be estimated by curve-fitting using non-linear regression (first order reappearance curve), i.e. a least squares regression analysis. The time in days to regain 50 and 90% of the eliminated amount was derived from \( k \), since \( T_{50\%} = 0.69/k \) and \( T_{90\%} = 2.30/k \). When in this first-order model the lipoprotein pool is acutely depleted, the subsequent asymptotic recovery is characterised by a constant absolute inflow and a constant fractional outflow of lipoprotein [16,22,23,26]. The absolute recovery rate was calculated by multiplying the change in pool size with \( k \), and expressed relatively to body weight (mg/kg/day). For estimation of the size of the removed pools, plasma volumes were calculated with the formula: 

\[ V_{PLASMA} = (1 - \text{hematocrit}[l/l]) \times \text{weight}[kg]/13. \]

Time-averaged concentrations (\( C_{AVG} \)) were measured in each patient by integration of the area under the rebound curve. Since \( C_{AVG} \) is determined under first-order conditions by \( C_{MAX} \) and \( C_{MIN} \) and a constant \( a \) in the formula \( C_{AVG} = aC_{MAX} + (1 - a)C_{MIN} \), we were able to calculate \( a \) for all procedures from \( a = (C_{AVG} - C_{MIN})/(C_{MAX} - C_{MIN}) \) [39]. To investigate whether time-averaged levels of total cholesterol, LDL cholesterol, Lp(a), and apo B could be estimated correctly by using this simple formula, we used a median \( a \) for all four parameters. Time-averaged levels calculated with this formula were then compared with those measured by integration under the curve.

Analyses were performed with procedures available in the software package of SPSS (SPSS Inc., Chicago, IL). Multivariate analysis of variance (ANOVA), followed by \( t \)-tests were used for normally distributed data, whereas Wilcoxon’s signed ranks tests were used for differences in means of not normally distributed data. For measurements of agreement the Pearson product-moment correlation coefficient was used. A two-sided \( P \)-value of less than 0.05 was considered to be significant. Results are expressed as means \( \pm \) SD, unless otherwise indicated.

3. Results

3.1. Acute reductions in plasma lipids and lipoproteins

During biweekly treatment with LDL-apheresis and simvastatin lipid and lipoprotein concentrations were reduced to the pre-treatment levels (\( C_{MAX} \)), as shown in Table 1. LDL-apheresis acutely reduced plasma total cholesterol and triglycerides to the same extent, 61 and 62%, respectively, whereas LDL cholesterol, apo B, and Lp(a) were lowered further and also to the same extent by an average of 77, 75, and 76%, respectively. HDL cholesterol levels were not influenced by LDL-apheresis. Immediate post-treatment levels (\( C_{MIN} \)) of all apo B-containing lipoproteins were below 10% limit values of a normal population, as shown in Table 1. The mean removed amounts in grams by a single apheresis are shown in Table 2. A good correlation was found between the removed amounts of lipids and lipoproteins and the initial amount in total plasma volume, with \( r \) ranging from 0.96 to 0.99 (\( P < 0.001 \)) for total cholesterol, triglycerides, LDL cholesterol, apo B, and Lp(a). This is shown exemplarily for LDL cholesterol in Fig. 1, based on five consecutive procedures in all 20 patients. Generally, this indicates that the higher the plasma concentration of LDL cholesterol, the higher is the removed mass, given a constant treated plasma volume of 5000 ml.
3.2. Description of the rebound curves

The recovery after LDL-apheresis of the various lipoproteins was different. Triglycerides recovered rapidly, reaching 83% of the pre-treatment levels within 1 day after treatment. Therefore, time-averaged levels of triglycerides were not significantly different when compared to pre-treatment concentrations, 1.69 ± 0.73 versus 1.76 ± 0.77 mmol/l, respectively (P = 0.21). The recovery of the other lipid and lipoproteins was much slower. The time for recovery of 50% of the removed amount (T_{50%}) was between 2–4 days, followed by a successive slower rise indicated by the time for recovery of 90% of the eliminated amount (T_{90%}) (Table 2). The median values for the first-order turnover constant k of total cholesterol, LDL cholesterol, apo B, and Lp(a) were 0.23, 0.18, 0.20, and 0.31, respectively, showing the fastest recovery of apo B and an intermediate position for total cholesterol. The rebound curves of Lp(a) and LDL cholesterol, when expressed as a proportion of the removed amount of lipoprotein, were not significantly different (Fig. 2). The absolute recovery rate (Table 2) was lowest for Lp(a), subsequently followed by LDL cholesterol (compared with Lp(a); P = 0.03), total cholesterol (compared with LDL: P = 0.006), and apo B (compared with total cholesterol; P = 0.08). Pearson’s correlation coefficients between the removed amount and the absolute recovery rate were as follows: total cholesterol r = 0.45 (P = 0.04), LDL cholesterol r = 0.64 (P = 0.002), Lp(a) r = 0.74 (P = 0.001), and apo B r = 0.17 (P = 0.48). This generally indicated a higher absolute recovery rate of LDL cholesterol and Lp(a) in patients with high pre-treatment levels.

3.3. Time-averaged concentrations

Time-averaged (C_AVG) lipid and lipoprotein levels, calculated by integrating beneath the rebound curve, are shown in Table 1. These concentrations could be uniformly estimated by the formula mentioned in the method section. Mean levels of a for total cholesterol, LDL cholesterol, apo B, and Lp(a) were 0.72 ± 0.09, 0.70 ± 0.09, 0.78 ± 0.10, and 0.68 ± 0.11, respectively. The application of the overall median z = 0.73 showed very good correlations between both methods of calculation of C_AVG for all four parameters, with correlation coefficients r ranging from 0.96 to 0.99 (P < 0.001).

3.4. Measurements of plasma lathosterol and sitosterol levels

Pre- and post-treatment lathosterol and sitosterol concentrations, only measured in 12 patients, are shown in Table 3. Pre-treatment levels and ratios to cholesterol were high, when compared to normal ranges in men with the same age and cholesterol levels [31], indicating an overall increased whole-body cholesterol.

### Table 1
Change in plasma lipids and lipoproteins

<table>
<thead>
<tr>
<th>Description</th>
<th>TC (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>LDL-C (mmol/l)</th>
<th>HDL-C (mmol/l)</th>
<th>Lp(a) (mg/dl)</th>
<th>Apo B (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_MAX</td>
<td>5.36 ± 1.22</td>
<td>1.76 ± 0.77</td>
<td>3.48 ± 1.20</td>
<td>1.07 ± 0.21</td>
<td>60.6 ± 67.5</td>
<td>1.53 ± 0.37</td>
</tr>
<tr>
<td>C_MIN</td>
<td>2.07 ± 0.50</td>
<td>0.46 ± 0.20</td>
<td>0.79 ± 0.44</td>
<td>1.06 ± 0.19</td>
<td>14.2 ± 14.6</td>
<td>0.39 ± 0.13</td>
</tr>
<tr>
<td>% Change</td>
<td>−61 ± 5*</td>
<td>−62 ± 9%*</td>
<td>−77 ± 6%*</td>
<td>−2 ± 7</td>
<td>−76 ± 13%*</td>
<td>−75 ± 6%*</td>
</tr>
<tr>
<td>C_AVG (AUC)</td>
<td>4.36 ± 0.95</td>
<td>1.69 ± 0.73</td>
<td>2.47 ± 0.97</td>
<td>1.07 ± 0.20</td>
<td>53.7 ± 49.4</td>
<td>1.28 ± 0.27</td>
</tr>
<tr>
<td>C_AVG (formula)</td>
<td>4.44 ± 1.16</td>
<td>2.57 ± 1.08</td>
<td>–</td>
<td>–</td>
<td>57.9 ± 57.2</td>
<td>1.24 ± 0.35</td>
</tr>
</tbody>
</table>

* Data represent mean ± SD (n = 20). TC, total cholesterol; TG, triglycerides; L(H)DL-C, low (high) density lipoprotein cholesterol; Lp(a), lipoprotein(a); apo B, apolipoprotein B; C_MAX and C_MIN, levels immediately before and after five consecutive aphereses; C_AVG, time-averaged levels, as measured by integration of the area under the rebound curve (AUC) or estimated by a formula (see text); % change versus C_MAX; *, P < 0.001 (t-test or Mann–Whitney U-test where appropriate).

### Table 2
Kinetics of recovery after a single LDL-apheresis during treatment with simvastatin

<table>
<thead>
<tr>
<th>TC</th>
<th>LDL-C</th>
<th>Lp(a)</th>
<th>ApoB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removed pool (g)</td>
<td>4.51 ± 1.72 (4.03)</td>
<td>3.54 ± 1.62 (3.03)</td>
<td>2.04 ± 2.18 (1.53)</td>
</tr>
<tr>
<td>k (Pools/day)</td>
<td>0.24 ± 0.08 (0.23)</td>
<td>0.19 ± 0.08 (0.18)</td>
<td>0.24 ± 0.18 (0.20)</td>
</tr>
<tr>
<td>T_{50%} (days)</td>
<td>3.2 ± 1.0 (3.0)</td>
<td>4.3 ± 2.2 (4.0)</td>
<td>3.9 ± 1.9 (3.5)</td>
</tr>
<tr>
<td>T_{90%} (days)</td>
<td>10.5 ± 3.5 (10.0)</td>
<td>14.4 ± 7.4 (13.2)</td>
<td>12.9 ± 6.3 (11.8)</td>
</tr>
<tr>
<td>Abs. Recovery rate (mg/kg/day)</td>
<td>12.9 ± 4.5 (12.7)</td>
<td>8.2 ± 4.7 (7.4)</td>
<td>4.7 ± 4.9 (3.5)</td>
</tr>
</tbody>
</table>

* Data represent mean ± SD and median levels between brackets (n = 20). TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; Lp(a), lipoprotein(a); apo B, apolipoprotein B; k, first-order turnover constant; T_{50%} and T_{90%}, time for resynthesis of 50% and 90% of the eliminated concentration; abs. recovery rate, absolute recovery rate.
Fig. 1. Low density lipoprotein (LDL) cholesterol removed (g) by LDL-apheresis using dextran-sulfate cellulose columns in relation to the circulating amounts (g) in five consecutive procedures of all 20 patients.

synthesis in these patients on long-term apheresis and a cholesterol synthesis inhibitor. After a single apheresis a significant reduction of $67 \pm 14\%$ ($P < 0.001$) in lathosterol and $57 \pm 9\%$ ($P < 0.001$) in sitosterol levels were found. However, when expressed relatively to cholesterol no significant changes in plasma total lathosterol and sitosterol levels were observed immediately after apheresis and in days following apheresis (Table 3). Moreover, multivariate ANOVA of the recovery curves in days after apheresis of both lathosterol to cholesterol ratio and sitosterol to cholesterol ratio showed no statistically significant changes compared to pre-treatment levels, $P = 0.44$ and $0.37$, respectively, indicating no further increase in cholesterol synthesis nor in cholesterol absorption rate in the days post-apheresis.

4. Discussion

The aim of the present study was to examine acute changes induced by a single apheresis procedure during chronic treatment. It was demonstrated that LDL-apheresis induced substantial reductions in plasma lipids and lipoproteins, more than has been shown by others using DSC columns, immunoabsorption, or HELP (Heparin Extracorporeal LDL Precipitation) [7–13]. Dependent on the; initial levels, apheresis selectively removed large amounts of apo B-containing lipoproteins from the body, including VLDL, roughly 5–6% of the total exchangeable cholesterol body stores [40]. The post-treatment rebound of serum triglycerides was very fast, reaching pre-treatment values within 1–2 days, whereas the recoveries of apo B, total cholesterol, LDL cholesterol, and Lp(a) during concomitant therapy with simvastatin ranged between 8 and 13 days, and were not associated with a post-treatment increase in whole-body cholesterol synthesis or intestinal cholesterol absorption. Long-term turnover studies using radio-labelled cholesterol have suggested the existence of three body pools of cholesterol [40]. After depletion of the rapid exchangeable pool, represented by e.g. plasma

Table 3

Mean plasma total lathosterol and sitosterol levels immediately before, after, and in days following LDL-apheresis during chronic treatment with simvastatin

<table>
<thead>
<tr>
<th></th>
<th>Lathosterol (mmol/l)</th>
<th>Latho/chol (mmol/mmol)</th>
<th>Sitosterol (mmol/l)</th>
<th>Sito/chol (mmol/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>16.03 ± 7.07</td>
<td>3.24 ± 1.49</td>
<td>14.24 ± 7.30</td>
<td>2.77 ± 0.97</td>
</tr>
<tr>
<td>After</td>
<td>5.91 ± 2.83*</td>
<td>3.16 ± 1.42</td>
<td>6.11 ± 3.08*</td>
<td>3.03 ± 1.13</td>
</tr>
<tr>
<td>Day 1</td>
<td>7.49 ± 5.02*</td>
<td>2.95 ± 1.83</td>
<td>6.60 ± 2.53*</td>
<td>2.92 ± 1.50</td>
</tr>
<tr>
<td>Day 2</td>
<td>9.40 ± 5.48*</td>
<td>3.10 ± 1.78</td>
<td>9.11 ± 3.05*</td>
<td>3.06 ± 0.72</td>
</tr>
<tr>
<td>Day 3</td>
<td>12.24 ± 6.84*</td>
<td>3.21 ± 1.97</td>
<td>10.20 ± 6.60*</td>
<td>2.75 ± 0.93</td>
</tr>
<tr>
<td>Day 7</td>
<td>17.1 ± 6.81</td>
<td>3.36 ± 1.43</td>
<td>13.80 ± 7.56</td>
<td>2.97 ± 1.24</td>
</tr>
<tr>
<td>Day 10</td>
<td>15.96 ± 5.84</td>
<td>3.27 ± 1.37</td>
<td>13.02 ± 4.78</td>
<td>2.76 ± 0.77</td>
</tr>
</tbody>
</table>

* Data represent mean ± SD of two procedures in 12 patients. Latho/chol, lathosterol to cholesterol ratio; sito/chol, sitosterol to cholesterol ratio. Normal values in a sex-, age-, and cholesterol-matched population: lathosterol 7.43 ± 2.84 mmol/l, sitosterol 6.78 ± 2.88 mmol/l ($n = 158$) [50]. Differences versus ‘before’: *, $P < 0.001$; #, $P < 0.01$ ($t$-test).
lipoproteins, erythrocytes, liver, and intestines, recuperation of this pool is mediated by input via an intermediate exchangeable pool (e.g. skin and adipose tissues) and a slowly exchangeable pool (e.g. skeletal muscles and arterial wall). In addition, cholesterol homeostasis will also be corrected by increased cholesterol synthesis and intestinal absorption. Using stable isotope methodology, Arends et al. [26] have shown no increase in the absolute hepatic VLDL apo B production as well as LDL apo B production in normal volunteers after a single LDL-apheresis. Among others [24], these latter authors plausibly showed a constant absolute apo B synthesis versus a constant fractional catabolic rate independent of the change in pool size post-apheresis, which concords with a simple one-compartment model as assumed by Apstein et al. [16]. Analyses of such models reduces complexity, but have also been shown to yield similar results as multi-compartment models [41]. Moreover, values for \( k \) calculated as described in the method section using a simple non-linear model of asymptotic regression, have been shown to be comparable to those measured in steady state conditions during turnover studies with radio-labelled isotope [16,42].

Comparison of the absolute recovery rates and the recovery half-times (\( T_{50\%} \)) clearly showed the highest rebound for apo B and total cholesterol. This must be explained by the very fast recovery of triglycerides, in particular VLDL particles. It has been reported that the absolute production rate of VLDL apo B is twice that of LDL apo B and returns to baseline 16 h after apheresis [26]. The major apolipoprotein of LDL, apo B-100, is predominantly synthesised in the liver and enters the circulation as a component of VLDL [43,44]. Conversion of VLDL to LDL and thus transfer of apo B to LDL takes place through lipolysis. Since we did not observe a post-treatment increase in intestinal absorption, i.e. sitosterol to cholesterol ratio, the fast increase in triglycerides after apheresis reflects de novo VLDL synthesis. This explains that \( T_{50\%} \)-values for apo B and total cholesterol are lower than for LDL, because they also reflect the rapid increase in VLDL particles, which are not present in the LDL fraction.

We did not observe a different rebound between LDL cholesterol and Lp(a), as has been shown by others [10,47]. Median values for constant \( k \) of LDL cholesterol in our study were lower than what has been shown by others, ranging between 0.22 and 0.23, which is probably associated with the more effective inhibition of HMG-CoA reductase caused by simvastatin [17,47]. As indicated by a median \( k \)-value of 0.14 for Lp(a), Armstrong et al. [45] observed a slower rebound of Lp(a) in comparison to LDL, whereas Koizumi et al. [47] found a faster recovery for Lp(a). However, the recovery curves of the latter author were only based on three time points. Taking into account the skewness of their data, the more appropriate median figure for \( k \) of Lp(a) in this latter study of 0.19 indicates rather a slower rebound when compared to their median \( k \) for LDL of 0.22. Therefore, there is no reason to believe that the rebound of Lp(a) is faster than that of LDL cholesterol, as indicated by our study.

Body stores would be quickly depleted if not replaced by absorption of dietary cholesterol or newly synthesised cholesterol. Parker [19] and Pfohl et al. [20] have shown evidence for transiently increased endogenous cholesterol synthesis after LDL-apheresis. Only recently, data has been shown indicating a threshold effect: reduction of LDL cholesterol to levels below 1.0 mmol/l induces an upregulation of the cholesterol biosynthesis in normocholesterolemic subjects [48,49]. In agreement with Gylling et al. [25], however, we did not find an increase in cholesterol synthesis nor in intestinal absorption, although in our study LDL cholesterol was lowered to 0.79 mmol/l immediately after apheresis. These different observations may be explained by the fact that we measured the rebound during ongoing treatment with biweekly apheresis accompanied with simvastatin treatment, whereas other investigators have analysed the recovery of lipids and lipoproteins after one single procedure without concomitant treatment with a cholesterol synthesis inhibitor [19,26,45–49]. Indeed, we observed high pre-treatment ratios for plasma lathosterol and sitosterol relative to cholesterol as compared to a sex-, age-, and cholesterol-matched population [50], indicating an upregulated cholesterol synthesis and intestinal absorption before the start of the analysis of the rebound. Since it has been shown that long-term therapy with HMG-CoA reductase inhibitors induced a reduction or no change in the rate of whole-body cholesterol synthesis [51,52], the lack of a further transient increase in cholesterol synthesis post-apheresis in our study may either be explained by the yet increased rate of synthesis due to the many foregoing procedures or by the concomitant treatment with simvastatin. In this latter situation, however, this drug in the dosage of 40 mg per day did not prevent chronic increase in cholesterol biosynthesis due to aggressive LDL-apheresis. So, more aggressive lipid lowering by LDL-apheresis and simvastatin to post-treatment LDL cholesterol levels below 1 mmol/l caused an overall increase in cholesterol biosynthesis, however, without transient post-apheresis increments. The continuous rise and fall of plasma lipoproteins necessitates the estimation of the time-averaged levels, since the mean of pre- and post-treatment concentrations overestimates effective plasma level [10].

We showed that the estimation of time-averaged levels of total cholesterol, LDL cholesterol, apo B, and Lp(a) could be accurately performed by a simple uniform formula using only pre- and post-treatment levels, \( C_{AVG} = C_{MIN} + 0.73(C_{MAX} - C_{MIN}) \), notwithstanding the differences in post-treatment rebound. Theoreti-
cally, this formula is not specific for the conditions we
used during the study, i.e. biweekly procedures and a
-treated plasma volume of 5000 ml. This should, how-
ever, be evaluated first before this formula is used
under different conditions.

In conclusion, our study showed that chronic, aggres-
sive lipid lowering with LDL-apheresis stimulated
cholesterol biosynthesis, which was not prevented by
concomitant treatment with simvastatin. Biweekly pro-
cedures, however, were not followed by a transient
further increase in cholesterol absorption or whole-
body synthesis. The fast rebound of the VLDL pool
determined a quicker recovery of total cholesterol and
apo B, whereas the return to pre-treatment levels of
Lp(a) and LDL cholesterol in 12 and 13 days, respec-
tively, showed that biweekly apheresis procedures,
treating 5000 ml of plasma, is an acceptable strategy.

Acknowledgements
This work was supported by the Dutch Heart Foun-
dation (grant 90.065). We gratefully acknowledge the
expert technical assistance of M. Hectors, P. van Heijst,
and H. Hak-Lemmers.

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